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Advances in Dendritic Cell-Based Vaccine of Cancer

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ABSTRACT

Dendritic cells (DCs) are potent antigen presenting cells that exist in virtually every tissue, and from which they capture antigens and migrate to secondary lymphoid organs where they activate naïve T cells. Although DCs are normally present in extremely small numbers in the circulation, recent advances in DC biology have allowed the development of methods to generate large numbers of these cells in vitro. Because of their immunoregulatory capacity, vaccination with tumor antigen-presenting DCs has been proposed as a treatment modality for cancer. In animal models, vaccination with DCs pulsed with tumor peptides, lysates, or RNA or loaded with apoptotic/necrotic tumor cells could induce significant antitumor CTL responses and antitumor immunity. However, the results from early clinical trails pointed to a need for additional improvement of DC-based vaccines before they could be considered as practical alternatives to the existing cancer treatment strategies. In this regard, subsequent studies have shown that DCs that express transgenes encoding tumor antigens are more potent primers of antitumor immunity both in vitro *and* in vivo *than DCs simply pulsed with tumor peptides. Furthermore, DCs that have been engineered to express certain cytokines or chemokines can display a substantially improved maturation status, capacity to migrate to secondary lymphoid organs* in vivo, *and abilities to stimulate tumor-specific T cell responses and induce tumor immunity* in vivo. *In this review we also discuss a number of factors that are important considerations in designing DC vaccine strategies, including (i) the type and concentrations of tumor peptides used for pulsing DCs; (ii) the timing and intervals for DC vaccination/boosting; (iii) the route of vaccination; and (iv) the maturation status of the DCs. Taken together, the available data on DC vaccination portends bright prospects for this approach to tumor immune therapy, either alone or in conjunction with other therapies.*

Key words: Dendritic cell, tumor antigen, lysate, RNA, engineered dendritic cell, antitumor immunity

INTRODUCTION

Mature dendritic cells (DCs) are characterized by having numerous membrane processes that take the form of dendrites, pseudopods, or veils. As the most potent antigen-presenting cells (APC) for primary immune responses, they are also characterized by displaying high levels of major histocompatibility complex (MHC) class II antigens, and various adhesion and costimulatory molecules (e.g., CD11a, CD11b, CD11c, and CD54) on their surface. As with other APCs, the costimulation-associated molecules CD80, CD86, and CD40 are expressed on mature DCs, and CD83 is now recognized also as specific marker of mature human DCs. But DCs can process antigens via the classical pathway, whereby endogenous antigens are delivered via proteosomes into

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the MHC class I compartment, and exogenous antigen via endocytic lysosomes into the MHC class II compartment. DCs also posses an alternative pathway of antigen processing and can route exogenous antigen into the MHC class I pathway through a mechanism known as cross-priming. They can also utilize molecular chaperones, such as heat shock protein (hsp), to deliver antigens via the class I pathway.[1] Murine splenic DCs can express CD4 or CD8 markers. It is known that various subsets of DCs exist in humans and mice and that they can play differing roles in the regulation of immune responses.[2–4] DCs have also been shown capable of inducing strong antitumor immunity.[5]

DCs migrate as precursors from bone marrow into various organs, where they usually reside in an immunologically inactive state, during which they possess the ability to efficiently endocytose and process antigens.[6] They can be activated however by an array of inflammatory stimuli, after which they undergo a differentiation process that down-regulates further antigen-processing capabilities, but enhances their expression of MHC, co-stimulatory, and other molecules important for successful antigen presentation. They then migrate to regional lymphoid organs to interact with naïve T cells.[7] This capacity of stimulated DCs to migrate into T cell-rich areas of lymph nodes is key to the successful induction of protective immunity.[6] Recent studies have demonstrated that chemokines play critical roles in this directed migration. Immature DCs respond to inflammatory chemokines (e.g., MCP-1, MIP-1α, MIP-3α, and RANTES) via their CCR1, CCR2, CCR5, CCR6, and CXCR1 receptors and are thereby drawn to sites of inflammation, whereas mature DCs respond via the CCR-7 receptor to the MIP-3β and SLC expressed strongly in the lymph nodes.[8–10]

Based upon the availability of recombinant cytokines essential for DCs growth and maturation (e.g., GM-CSF, IL-4)[11,12] bone marrow (BM)-derived DCs can now be generated in large numbers simply by culturing BM cells in GM-CSF/IL-4.[13] However, the phenotype of the DCs so-generated is critically dependent upon the precise culture conditions. BM cells cultured with low doses of GM-CSF (2 ng/mL) alone or high doses of GM-CSF and IL-4 (each, 10–20 ng/mL) can differentiate into immature DCs (DC_{IMAT}) or relatively mature DCs (DC_{RMAT}), respectively.[11] DC_{IMAT} lack expression of MHC Class II and costimulatory molecules, while DC_{RMAT} express intermediate levels of MHC class II and CD80 and low levels of CD40. DC_{RMAT} can be induced to full cellular maturity (i.e., DC_{MAT}) by exposure to stimuli such as LPS, DNA, TNF, hsp or agonistic anti-CD40 antibodies;[7,14–16] they then express yet higher levels of MHC class II, CD40, CD80, as well as other maturation markers. Injection of Flt3–ligand into humans or mice leads to a substantial expansion of the total DC population,[17–19] while adenoviral transduction of DC precursors with a GM-CSF gene renders exogenous GM-CSF supplementation unnecessary for the production of mature DCs in culture.[20] Such technical advances, combined with an increasing knowledge of the powerfully important roles DC can have in the initiation of immune responses, have provided a compelling impetus for pursuing DCs-based immunotherapies for cancer. The purpose of this review is to briefly summarize the recent progress in this field. By drawing inferences from animal models and supporting these with empirical data from clinical trials with human DC (see Table 1), we will highlight the promises of the new generation of DC-based cancer vaccines, including tumor antigen-presenting, and genetically-engineered immunostimulatory DCs.

INDUCTION OF ANTI-TUMOR IMMUNITY WITH TUMOR ANTIGEN-PULSED DCs

Tumor-antigen-pulsed DCs have been demonstrated to induce the development of MHC-class I- and class II-specific T cell responses *in vitro* and *in vivo*. DCs pulsed *in vitro* with peptide antigen and subsequently given to animals can induce antigen-specific, cytotoxic T lymphocyte (CTL)-mediated protection against lethal tumor challenges, and can even induce regression of established tumors.[5] While administration of tumor peptides alone can lead to peptide-specific CTL tolerance, delivery of the same peptide by DCs can lead to dramatic immunostimulation.[21] As shown in Table 1, delivery by DCs of an array of human and mouse tumor antigens/peptides can lead to marked antitumor immune responses. Specifically, MHC-restricted synthetic tumor-associated peptides, such as melanoma-related antigen,[22–28] endogenous retroviral gene products gp70/p15E,[29] carcinoembryonic antigen (CEA),[30,31] folate binding protein (FBP),[32] prostate-specific membrane antigen (PMSA),[33] survivin,[34] MUC-1,[35] HER2/neu,[36–38] and idio-

Table 1. DC-Based Vaccine of Cancer

DC vaccine	*Mouse*	*Human*
Peptide pulsed	Mut 1[5], OVA[5], gp70/p15E[29], HER2/neu[35], bcr-abl[43], E7[5,47], Id-KLH[145], TRP-2[146]	MAGE-1/3, Melan-A, gp100, tyrosinase[22-28], CEA[30,31], FBP[32], PMSA[33], Survivin[34], MUC-1[38], HER2/neu[36,37], Idiotype[39-42], bcr-abl[44], E7[45,46]
Lysate pulsed	mammary carcinoma[49], hepatocellular carcinoma[50], murine renal carcinoma[51], gliomas[52], B16 melanoma[146], MCA105 sarcoma cell, mammary adenocarcinoma TS/A[163]	melanoma[23], lung tumor[49], pancreatic carcinoma[53], brain tumor[54,55], ovarian cancer[56], parathyroid carcinoma[57], breast tumor[58], uterine serous papillary cancer[59], renal cell carcinoma[60-63], pediatric solid tumor[64]
RNA pulsed	B16/F10.9 melanoma tumor[65], MUC-1[71]	CEA-positive colorectal cancer[65], CEA[66,68], HIV gag[70], PSA[72], colorectal cancer[74], bladder cancer[75], renal tumor[76]
Apoptotic/necrotic tumor cells	melanoma cells[79], BL6-10[94], leukemia cells[95], squamous cell carcinoma[96]	Jurkat T cell lymphoma[97]
Gene modified		
Tumor antigen genes	hMART-1[120], hTRP-2[117], PSA[108], Glioma cDNa[109], MAGE-1[131], p53[124,125], Hugp100[114]	HER2/neu[123], TRP-2[121], MUC-1[118], gp100[106]
Immunomodulatory molecule genes	IL-12[134], IL-12+HER2/neu[122], IL-2+MUC-1[105], TNF-α[131,132], IL-18[138], GM-CSF[127,128], SLC[141,142], lymphotactin[143,144], CD40 ligand[131,152,153]	

typic protein (Id)-derived peptides,[39–42] synthetic *bcr-abl* chimeric nonapeptide (GFKQSSKAL)[43,44] and a synthetic HPV 16 $E7_{[49-57]}$ peptide,[45–47] have been identified, delivered to DCs and used, more or less successfully, in animal studies or clinical trials.

Vaccination with DCs Bearing Specific Tumor Peptides

DCs pulsed with individual, or cocktails of, peptides such as MART-1/Melan A, melanoma antigen (MAGE)-1, MAGE-3, gp100,[22] or tyrosinase have been used to treat melanoma tumors, such that antigen-specific immunity has been induced by DC vaccination. Objective responses have been evident in 5 of 16 treated patients, with 2 undergoing complete, and 3 partial, regression of metastases in skin, lung, pancreas, or other soft tissues.[23–25] In another trial, 16 patients with metastatic stage IV melanoma were treated intravenously (i.v.) with PBMC-derived DCs that had been pulsed with modified tyrosinase $368–376_{(370D)}$ and gp100 $209–217_{(210M)}$ peptides; 10 of these received one cycle, and 6 others two cycles, of treatment. One patient experienced a complete remission of lung and pleural disease after two cycles of DCs therapy.[26] MAGE actually comprise a family of tumor-specific antigens expressed in various tumors, including bladder cancers, gastrointestinal carcinomas and melanoma, but not in normal tissues (except for the testis). Thus, autologous DCs that have been pulsed with the human leukocyte antigen (HLA)-A24–specific MAGE-3 epitope peptide (IMPKAGLLI) have been used for subcutaneous (s.c.) treatment of bladder cancer or gastrointestinal carcinoma patients. In these cases, antigen-specific CTL response were significantly higher when induced by the IMPKAGLLI-pulsed autologous DCs than following either tumor cell or untreated DC (i.e., not pulsed with tumor peptides) treatment alone; three of four IMPKAGLLI-DC treated patients showed significant reductions in the sizes of lymph node and/or liver metastases.[27,28]

Overexpression of the proto-oncogene epidermal growth factor receptor-2 (HER2/neu) occurs in 20–30% of patients with breast cancer, and is considered indicative of a poor prognosis. Thus, the presence of a detectable immune response to HER2/neu in some patients has suggested that this oncogene should perhaps be explored as a potentially key target for vaccine therapy, partic-

ularly since proto-oncogene expression/activation is largely, or exclusively, restricted to transformed cells and their precursors. The overexpression of HER2/neu is often detected in adenocarcinomas such as breast, ovarian, lung, and gastric cancers. Experimentally, weekly vaccination of BALB/c mice with syngeneic HER2/neu$_{p780-788}$ peptide-pulsed DCs has led to suppressed growth of previously transplanted HER2neu-expressing syngeneic tumors. It is encouraging that this HER2/neu$_{p780-788}$ peptide is common to both murine and human HER2/neu. And that CD8$^+$ CTL clones specific for HER2/neu-expressing tumor cells have been established *in vitro* from HLA-A2402–positive human peripheral blood lymphocytes, by repeated challenge with peptide-pulsed autologous DCs. Thus, HER2/neu$_{p780}$ may function as a tumor rejection antigen/peptide, at least in HLA-A2402–positive individuals;[36] HER2/neu$_{p63-71}$,[37] and HER-2/neu$_{[9(754)]}$[38] are alternate peptides also capable of inducing specific CTL.

Just as with HER2/neu with melanoma and other tumors, the epithelial mucin MUC1 is overexpressed on many epithelial malignancies, some B-cell lymphomas and multiple myelomas, and on primary AML blasts. It has been shown that primary AML blasts that constitutively express both MUC1 and HLA-A2 are efficiently and specifically killed by CTLs from HLA-A2$^+$ healthy donors previously vaccinated with DCs that had been pulsed with MUC1–derived HLA-A2–restricted peptides. This suggests that MUC1–derived peptides could also potentially be used for immunotherapeutic approaches to some cancers.[35] Indeed, patients with advanced breast and ovarian cancer can be efficiently vaccinated with autologous DCs pulsed with HER-2/neu- or MUC1–derived peptides; in 5 of 10 patients, peptide-specific CTLs could be detected in their peripheral blood. The major CTL responses lasted for more than 6 months and were associated with the HER-2/neu-derived E$_{75}$ or the MUC1–derived M1.2 peptide, which suggests that these might be immunodominant peptides. These results confirm that vaccination with DCs pulsed with a single tumor antigen can induce effector immune responses in patients with breast and ovarian cancer.[48]

The tumor vaccine approach of employing defined peptides to pulse DC relies on an accurate identification of the correct peptides to be utilized. These are chosen based on predicative algorithms to identify peptides with high binding affinity for HLA molecules, most commonly the HLA*A0201 alleles. Those peptides with demonstrated binding to HLA*A0201 are then assessed for their immunogenicity based upon their abilities to generate CTL *in vitro*. Although this approach may simplify the vaccine discovery process by limiting the numbers of antigens/peptides to be targeted, it has its own disadvantages, including the fact that the treatable patient base is restricted to those individuals expressing a HLA haplotype with a capacity to bind the identified tumor peptide(s) (i.e., 30–40% of the population). A further limitation is that, by selectively using MHC class I-restricted peptides, one also ignores the important roles of MHC class II-restricted T helper cells in initiating and sustaining immune responses, including CTL responses.[1]

Vaccination Using DCs Loaded with Total Tumor Lysate Antigens

Vaccination strategies directed against a single tumor antigen peptide or epitope may be overly narrow in scope, with the immune system investing all of its effector resources in a single response. On the other hand, the use of whole tumor lysates as a source of antigen offers the potential advantage of inducing broad-spectrum T cell responses against multiple known, as well perhaps as unknown, tumor-associated antigens expressed within the tumor. As alluded to above, these might induce not only the critical baseline CTL responses, but also helper T cell responses important to a more complete realization of the full anti-tumor CTL potential. This approach also reduces the oftentimes very substantial efforts required to identify and generate individual peptides, and leaves the choice of peptides to the DC itself, which selects all peptides within the lysates that are capable of binding to its MHC molecules. Alternately, in principle one could acid-elute tumor peptides from loaded MHC class I molecules on the surface of tumor cells as another means of accomplishing this but, in practical terms, total tumor lysate approaches to loading DC for tumor vaccine strategies can be highly effective.

In two separate strains of mice with histologically distinct tumors, s.c. injection of tumor lysate-pulsed DCs has been shown to effectively prime for subsequent rejection of lethal challenges with viable parental tumor cells, and also to reduce significantly the number of metastases subse-

quently established in the lungs of these animals.[49] Experimental tumor lysate-DC vaccination has also been effective against hepatocellular carcinoma BNL1MEA.7R.1 (BNL),[50] murine renal cell carcinoma,[51] and syngeneic GL261 gliomas[52] in mice. Similarly, DCs that have been pulsed with lysates from pancreatic carcinoma cells,[53] malignant brain tumors,[54,55] or ovarian cancers[56] can induce tumor antigen-specific CTL responses *in vitro*. Most important, DC-tumor lysate vaccination of cancer patients has also been shown to be beneficial in the treatment of malignant melanoma,[23] parathyroid carcinoma,[57] advanced breast [58] and uterine serous papillary cancer,[59] renal cell carcinoma (RCC)[60–63] and solid tumors.[64] With the latter study, autologous monocyte-derived DCs were either pulsed with lysates of weakly immunogenic patient tumors or with the highly immunogenic antigen keyhole limpet hemocyanin (KLH), then the two DC populations were combined for vaccination of pediatric patients. The rationale for this approach was that the immunogenic KLH would, through bystander effects, also make the tumor antigens more immunogenic. Its safety was indicated by the fact that no obvious patient toxicity was observed. Significant regression of multiple metastatic sites was observed in one patient; in 5 of 10 patients their disease remained stable after treatment, and three of these (whom had had minimal disease at the time of vaccine therapy) remained tumor-free 16–30 months later.[64]

In one parathyroid carcinoma patient treated with tumor lysate-pulsed autologous DCs, augmented *in vitro* T cell proliferative responses and delayed type hypersensitivity (i.e., erythema and induration) responses to tumor lysate challenge were observed, suggesting that the treatments efficiently induced the generation of tumor lysate-specific memory T cells.[57] Seven glioblastoma multiforme patients and two anaplastic astrocytoma patients were given three biweekly vaccinations with autologous DCs loaded with tumor lysates; systemic CTL activity was detected in four of seven of these patients that were tested, while robust intratumoral cytotoxic and memory T-cell infiltration was detected in two of four patients who had again undergone surgery after vaccination.[54] These data indicate clearly that tumor antigen-laden DCs can be used safely to vaccinate cancer patients, and that these treatments can have beneficial effects in these subjects.

THERAPEUTIC USE OF TUMOR mRNA-PULSED OR TRANSFECTED DCs

Just as DCs can be treated with tumor peptides or lysates, they can be pulsed or transfected with tumor RNA. Successfully transfected DCs then translate the respective tumor proteins, with all of the epitopes they encode; when processed, these tumor antigens would also carry the advantage of having broader HLA specificities and thus permit the induction of CTL responses almost irrespective of the patient s HLA repertoire. A further advantage of using mRNA is that it can be isolated from murine tumor cell lines or from primary human tumor cells microdissected from frozen tissue sections, and amplified at will without loss of function.[65,66]

DCs transfected with tumor cell mRNA can stimulate potent CTL responses and engender protective immunity in tumor-bearing mice.[65] Tumor mRNA can be efficiently transfected into DC, resulting in superior translation product yields in these cells relative to other professional APCs. Most researchers have used mRNA/liposome complexes to transfect DCs, although more efficient mRNA delivery may be achieved by electroporation when using human hematopoietic cells.[67,68]. Such mRNA-mediated delivery to DCs of encoded tumor antigens can induce potent primary T cell responses *in vitro*. This is largely because transfection of DCs with tumor mRNA delivers maturation/activation signals to the cells and mediates efficient delivery of antigenic peptides to MHC class I and II molecules. Thus, when used in anti-tumor vaccine strategies, this approach has the potential to potently induce tumor-specific effector T cell activation.[69,70] Investigations such as these provide a theoretical foundation for broadly applicable tumor treatments that do not require prior characterization of the relevant antigenic profile for each patient (i.e., the tumor peptides presented by their own HLA haplotype specificities) and would not be limited by the availability of tumor tissues for antigen preparation.[66]

As noted previously, MUC1 antigen is aberrantly overexpressed in human breast and other carcinomas. It has been shown that DCs that have been transfected with MUC1 mRNA induce CTL responses against MUC1–positive, but not MUC1–negative, tumor cells. Mice immunized with such MUC1–transfected DCs were protected against challenge with MC38/MUC1 tumor cells and,

furthermore, established MC38/MUC1 tumors were eliminated after vaccination. Moreover, vaccination with DCs transfected with MUC1 mRNA and IL-12 reversed immunologic tolerance to MUC1 in the animals and induced immunity against MUC1–positive tumors.[71]

Prostate-specific antigen (PSA) is a self-antigen expressed by both normal and malignant prostatic epithelium. In this tumor system too, autologous DCs transfected with PSA mRNA were capable of stimulating primary CTL response against PSA antigens *in vitro*. These PSA-specific CTL did not cross-react with kallikrein, an endogenous protein that shares significant homology with PSA, suggesting that autoimmune toxic effects may not represent a significant problem in this system.[72] Autologous DCs transfected with RNA amplified from microdissected prostatic tumor cells can also stimulate CTL responses against a broad set of unidentified and critical prostate-specific antigens. These results confirm the principle that, for prostate cancer patients, the use of tumor RNA-transfected DCs may represent a broadly applicable and clinically effective vaccine strategy that will not be limited by tumor antigen availability and which may minimize the risk of clonal tumor escape.[73] While autologous DCs pulsed with the immunogenic protein KLH and mRNA from advanced colorectal tumors have been used in clinical trials, to date dramatic clinical responses have not been observed over the brief follow-up periods extant since the treatments.[74] While DCs transfected with the urothelial carcinoma tumor mRNA can to some extent activate tumor-infiltrating lymphocytes, these cells are not highly effective at tumor cell killing; after one stimulation, their cytotoxicity achieved 35.7% at an effector:target ratio of 50:1.[75] Nevertheless, total tumor RNA-transfected DCs may represent a broadly applicable vaccine strategy to induce potentially therapeutic polyclonal T-cell responses in cancer patients.[76]

THERAPEUTIC EFFICACY OF DCs LOADED WITH NECROTIC OR APOPTOTIC TUMOR CELLS

DCs can readily take up soluble tumor antigens, such as proteins or immune complexes, but can also phagocytose dying (e.g., apoptotic or necrotic) tumor cells, and thereby induce protective antitumor immunity.[77,78] The recognition and uptake of apoptotic cells by DCs is regulated by specific receptors such as $\alpha V\beta 5$, CD36, or the phosphatidylserine receptor,[79,80] while uptake of necrotic cells is mediated by CD91, the receptor for heat shock proteins (hsp) exposed on these cells.[81,82] The advantages of using dying tumor cells as a source of tumor antigens are that: (i) DCs can present or cross-present both MHC class I and II epitopes of a defined tumor antigen,[83] or multiple tumor antigens (e.g., MAGE3 and gp100 of melanoma tumors);[79,84] and (ii) unlike the case with peptide-pulsed DCs, this approach is independent of HLA haplotype and can thus be applied equally to all patients.

The uptake of dying cells decidedly impacts DC maturation. Controversy exists, however, with respect to the best phagocytic target for optimal antitumor priming. According to the danger signal theory of Matzinger,[85] the immune system should be activated by internal injuries that signals such as the cellular necrosis that signals threats to the organism, but not by signals associated with more normal homeostatic processes, such as apoptosis.[86] Thus, it has been reported that DCs that have captured necrotic tumor cells induce immunological tolerance to the tumors,[87] although numerous other reports indicate that DC phagocytosis of apoptotic tumor cells can also induce effective antitumor immunity.[88,89] It has now been clearly demonstrated that the target cell's stage within the apoptotic process importantly affects the maturation of DCs engulfing the cells and thus also the antitumor immunity these cells can induce. Specifically, only tumor cells in the late, but not early, phases of apoptosis stimulate DC maturation and antitumor immunity.[90] Recent comparative analyses have shown that necrotic and late phase-apoptotic cells equally effectively trigger DC maturational changes that lead to the induction of antitumor immunity.[91] As noted above, DC phagocytosis of necrotic tumor cells is dependent on their expression of hsp, as is their subsequent maturation and thereby abilities to induce anti-tumor immunity.[91–93] We have shown that DCs that have phagocytosed tumor cells undergoing necrosis/apoptosis (as a result of exposure to lovastatin) undergo strong maturation responses, with up-regulated expression of proinflammatory chemokines and cytokines, and coimmunostimulatory molecules. These cells induce stronger protective immunity against tumor challenge in animal models than do DCs pulsed with MHC class I-restricted tumor peptides. This would be, at least in part, because the DCs that

phagocytosed effete tumor cells would present multiple MHC class I- and II-restricted tumor antigen epitopes.[94] DCs loaded with apoptotic leukemia cells are similarly protective against leukemia development in a mouse model system.[95] Furthermore, when combined with the delivery of IL-2, adoptive transfer of DCs that have been pulsed with apoptotic squamous cell carcinoma cells significantly suppresses tumor growth.[96]

Cross-presentation of tumor cell-derived antigens can be achieved by DCs loaded with killed tumor cells, which present MHC class I- and class II-restricted tumor peptides and thereby induce proliferation of autologous tumor-specific $CD8^+$ and $CD4^+$ T cells. Tumor cell-loaded DCs elicit expansion of CTLs specific for the tumor cells used for immunization. In cases where allogeneic cells are used, the induced CTL activity is not restricted to the alloantigens; and the induced allogeneic responses do not prevent activation of tumor-specific T cells. This finding thus opens the possibility of using allogeneic tumor cells as sources of antigen for tumor therapy.[97,98] In summary, DCs that have phagocytosed apoptotic/necrotic tumor cells appear to offer another new strategy in DC cancer vaccination.

IMPACT OF DC TRANSGENE EXPRESSION ON VACCINATION EFFICACY

Although the approaches described above are encouraging, they will not be applied in the majority of clinical cases largely because of the technical difficulties that accompany the preparation of such materials from human solid tumors. A new strategy, employing genetically modified DCs, recently has been developed for use in DC vaccination. The target genes transferred into the DCs fall into two categories, tumor-associated antigens (TAA) and immunomodulatory proteins such as cytokines or costimulatory molecules. Various methods have been used to introduce genes into DCs, including cationic lipids,[99] electroporation,[100] biolistic delivery (i.e., the gene gun),[101] complexes of plasmid DNA expression constructs with the cationic peptide CL22,[102] nonviral T7 vector,[103] viral vectors and adenoviral/polycation complexes.[104] The viral vectors that have been used up to now are poxvirus such as modified vaccinia Ankara (MVA),[105] retrovirus,[106] such as new lentiviral vectors derived from SIVmac251 (a simian immunodeficiency virus (SIV),[107] herpesvirus,[108] Semliki Forest virus (SFV),[109] influenza virosomes,[110] adeno-associated virus,[20,111] canarypox virus,[112] and adenovirus (AdV). Compared with the other vectors, the AdV vectors have the following advantages: (i) they have been studied widely and are very well-characterized (they comprise a long-standing model system for eukaryotic gene regulation); (ii) they have an intermediate size genome ($\approx$36 kilobases) that is capable of supporting large-sized gene inserts; (iii) they are easy to generate and manipulate; (iv) they are relatively stable and can be readily obtained in high titers (e.g., 10^{11}–10^{12} plaque-forming units/ml); (v) they exhibit high infectivity for a broad range of host species *in vitro* and *in vivo*, including non-dividing cells; (vi) because they do not require integration into the host cell genome, foreign genes so delivered are expressed episomally, and thus have low genotoxicity *in vivo*; (vii) the vectors can be introduced to different tissues via a variety of routes of administration; and (viii) no significant side effects have been reported following early clinical application or vaccination with AdV vectors.[113] Furthermore, replication-deficient Ad vectors represent a highly efficient and reproducible method of gene transfer into DCs, and one that enhances the maturation of these cells,[114] but does not markedly alter the phenotype of the cells or induce cytopathic effects.[115] Human monocyte-derived DC are permissive to AdV infection (independent of the coxsackie AdV receptor) at multiplicities of infection between 100 and 500.[115] Other notable observations are that DC infection with MART-1–carrying AdV (AdVMART1) does not significantly down-regulate the expression of cell surface MHC class I, even if the AdV E3 region is not deleted.[116] Also, immune responses to adenovirally-delivered transgenes are not impaired in mice that had been preimmunized against AdV (as might occur naturally in the general human population).[114] Thus, delivery of AdV-human tyrosinase-related protein-2 (hTRP2)-transduced DCs, but not recombinant AdV-hTRP2 virus by itself, was effective in inducing responses to hTRP2 in the presence of neutralizing anti-adenoviral antibodies.[117]

DCs Engineered to Express Tumor-Associated Antigens

The strategy of using genetically modified DCs expressing specific cancer antigens has several

advantages over using DCs simply pulsed with tumor antigen proteins or peptides. These include (i) a reduced need to assess the immunologic relevance of individual cancer-specific peptides (as long as the molecules transduced into the DCs are immunogenic), and (ii) the tumor proteins being constitutively-synthesized within the DC will allow for specific antigen presentation to T cells for longer periods without the concerns about the breakdown of peptide/MHC complexes. DCs that express tumor antigen transgenes are also more potent primers of antitumor immune response than their soluble antigen-pulsed counterparts, as determined both *in vitro* and in animal models.[118] Another advantage of using DCs engineered to express tumor antigens is their potential for generating $CD8^+$ T cell responses against multiple class I-restricted epitopes within the antigen, thereby eliciting a broad antitumor effector response.[118] Immunization through *ex vivo* transduction of DCs has been demonstrated as an effective approach to enhance antitumor immunity by activating $CD8^+$, as well as $CD4^+$, T cells.[119] As shown in Table 1, MAGE-1, glycoprotein 100 (gp100), MART-1, multiple melanoma tumor-associated antigen (TAA), hTRP2, p53, MUC-1 and other antigen genes have been used to transfect murine and/or human DCs and thereby induce tumor antigen-specific immune responses.

Replication-deficient recombinant AdV encoding human gp100 or MART-1 melanoma antigen have been used to transduce human DCs *ex vivo* in model systems for cancer vaccine therapy. Human DCs that have been transduced with a replication-defective E1–deleted AdVMART1 produce full-length MART-1 mRNA and protein. *In vitro* challenges with such DCs stimulated MART-$1_{(27–35)}$-specific tumor-infiltrating lymphocytes to synthesize IFN-γ and induced the generation of peptide-specific, MHC class I-restricted CTL within peripheral blood lymphocyte (PBL) from normal donors. A second generation E1/E4 region-deleted AdV (which harbors the CMV immediate-early promoter/enhancer and a unique E4–ORF6/pIX chimeric gene; Ad2) has also been developed. DC transduced with Ad2/gp100v2 can elicit tumor-specific CTL *in vitro* from patients bearing $gp100^+$ metastatic melanoma.[115] Similarly, transduction of an HLA-$A2^+$/MART-1^- cell line with AdVMART1 renders these cells sensitive to lysis by CTL specific for the MART-$1_{(27–35)}$ immunodominant peptide.[116] Mice vaccinated with AdVMART1–DCs generated protective responses to lethal tumor challenges with murine B16 melanoma cells. These responses were mediated by MHC class I-restricted, MART-1–specific CTL which produce high levels of IFN-γ when re-exposed to MART-1 *in vitro,* and kill their targets in a manner suggestive of perforin/granzyme-dependent lysis.[120]

An induction of tumor antigen-specific $CD4^+$ and $CD8^+$ T cells could be critical in the generation of an optimally effective immunotherapy for cancer. DCs transduced with gp100 induce the development of T cells recognizing three distinct HLA-A2–specific epitopes (i.e., $gp100_{(154–162)}$, $gp100_{(209–217)}$, and $gp100_{(280–288)}$) as well as $CD4^+$ T cells specific for a novel HLA-DR-beta1*0701–restricted gp100 epitope. The minimal determinant of this newly-discovered epitope was defined as $gp100_{(174–190)}$ (i.e., TGRAML-GTHTMEVTVYH). These observations confirm that retrovirally-transduced DCs can present multiple MHC class I- and class II-restricted tumor peptides, and thereby elicit robust immune responses against gp100.[106] Immunization of C57BL/6 mice with DCs transduced with Ad2 expressing human gp100 melanoma antigen (Ad2/hugp100) elicits the development of gp100–specific CTLs that can lyse syngeneic fibroblasts also expressing Ad2/hugp100, or B16 cells expressing endogenous murine gp100. This induction of gp100–specific CTLs is dependent on the presence of antigen-specific $CD4^+$ T cells. It gives rise to a long-term protection against lethal s.c. challenges with B16 cells, the level of which is dependent on the numbers of DCs used for the immunizations.[114,115] Interestingly, the potency of the DC-Adhugp100 vaccine appears to be a result of its ability to directly prime autoreactive $CD4^+$ cells through a process that does not require IL-12 and CD40 signals.[119] DC-based immunization can also afford partial protection against *established* B16 tumors, and this effect is improved by simultaneous immunization with DCs transduced with two melanoma antigens as opposed to only one.[114] Immunization via either i.v. or s.c. routes with cultured AdV-mTRP2–transduced DC could completely prevent the development of lung metastases following i.v. challenge of mice with B16 melanoma cells. T cell-depletion analyses indicated that here too the protective effects of immunization with AdV-mTRP2–transduced DC involved the participation of both $CD8^+$ and $CD4^+$ T-cells.[121]

Genetic immunization using DC transduced *ex vivo* with an AdV expressing the HER2/neu gene (AdNeuTK) can also induce immunity against a

breast tumor cell line overexpressing HER2/neu.[122] Subcutaneous immunization with this DC vaccine elicited protective immunity from tumor challenge in 60% of the treated animals, and CTL analyses demonstrated that the animals displayed specific cytotoxic activity against breast tumor cells, as well as syngeneic fibroblasts transduced with AdNeuTK. *In vivo* depletion studies demonstrated that, here too, both $CD4^+$ and $CD8^+$ T cells were required for effective immunity. In a therapeutic setting, these immunizations could cure mice with established tumors, with the efficacy of this effect being enhanced by also transducing the DCs to express murine IL-12 (Ad-VmIL-12).[122] Autologous $CD34^+$ hematopoietic progenitor-derived DC retrovirally-transduced with a HER2/neu gene elicited HER2/neu-specific $CD8^+$ CTL that lyse HER2/neu-overexpressing tumor cells in the context of distinct HLA class I alleles. The induction of both HLA-A2 and -A3–restricted HER2/neu-specific CTL was verified on a clonal level, and the presence of $CD4^+$ Th1 cells recognizing HER2/neu in the context of HLA class II was also documented. These HLA-DR-restricted $CD4^+$ T cells were cloned and found to release IFN-γ upon stimulation with DC that had been pulsed with HER2/neu extracellular domain. These data indicate that retrovirally-transduced DC expressing the HER2/neu molecule present multiple peptide epitopes and elicit HER2/neu-specific CTL and Th1 cells. Importantly, this method of stimulating HER2/neu-specific $CD8^+$ and $CD4^+$ T cells with retrovirally-transduced DC could also be successfully employed for *in vitro* generation of HER2/neu-specific CTL and Th1 clones from a patient with a HER2/neu-overexpressing breast cancer. This is, conceptually and practically, an important advance in DC vaccination therapy, for it provides a method for the generation and expansion of HER2/neu-specific, HLA-restricted CTL and Th1 clones *in vitro*. This will facilitate effective adoptive transfer of autologous HER2/neu-specific T cell clones into patients with HER2/neu-overexpressing tumors, without a need to define each tumor s immunogenic peptides.[123]

DCs engineered to express immunomodulatory molecules

As outlined in detail above, transduction of DCs with tumor antigens offers distinct advantages over simple pulsing of the cells with tumor peptides or lysates. Nevertheless, a major disadvantage is the critical nature of selecting an appropriate tumor antigen as the DC transduction candidate. MHC haplotype restrictions apply in the presentation of CTL and other epitopes, such that a substantial proportion of the candidate patient base may not be capable of responding to any one chosen peptide/antigen. An alternate strategy has been developed recently to augment the ability of DCs to present tumor antigens, and that is transducing them with expression vectors such that they constitutively express immunomodulatory proteins such as cytokines and chemokines. Thus, DCs genetically modified to express a T cell stimulatory cytokine, for example, could possess adjuvant-like properties useful in the treatment of any number of tumors, so long as sources of TAA were available. One could instead transduce the tumor cells themselves and count on their subsequent recruitment of APCs, but since DCs are themselves professional APC designed by nature to deliver their cytokines in precisely the correct context,[126] immunomodulatory gene-modified DCs (Table 1) would represent potentially more potent vaccines than similarly modified tumor cells.

GM-CSF is an essential *in vitro* growth and differentiation factor for DCs.[13] The fact that *in vivo* administration of GM-CSF augments primary immune responses suggests that enforced GM-CSF expression by DCs could perhaps further enhance the effectiveness of DC-based immunotherapy protocols. *In vitro*, the phenotype of bone marrow derived-DCs (BM-DCs) remains largely unaltered by GM-CSF gene transfection, but infection of the DC cell lines XS52–4D and XS106 with adenovirus-GM-CSF up-regulates their expression of MHC and costimulatory molecules, as well as their alloantigen or peptide antigen-presenting capacities. On the other hand, when used for *in vivo* immunizations, the antigen-presenting capacity of GM-CSF gene-transfected BM-DCs was greatly enhanced relative to mock-transfected or untreated DC, as determined by their abilities to induce primary immune responses to haptens, protein antigens, or tumor antigens. This increased efficacy correlated with an augmented migratory capacity of GM-CSF gene-transfected BM-DCs *in vivo*. These data thus suggest that GM-CSF gene transfection may be useful in improving DC-based vaccines currently under clinical investigation.[127,128]

TNF-α is another mediator known to contribute to both DC maturation and activation, and thus DCs generated by culture in GM-CSF and

exogenous TNF-α have been used for antitumor vaccination.[129,130] It is perhaps not surprising that vaccination of animals with TNF-α-transfected tumor peptide-pulsed DCs leads to increased survival times relative to animals simply treated with untransformed peptide-pulsed DCs.[131] More recently, we have shown that transfection of DCs with recombinant adenovirus AdV-TNF-α results in greater maturation of the DCs than occurs with control DCs cultured in levels of exogenous TNF-α equivalent to those secreted by the $DC_{AdVTNF\text{-}\alpha}$. Thus the $DC_{AdVTNF\text{-}\alpha}$ display up-regulated expression of proinflammatory cytokines (e.g., IL-1β and IL-18) and chemokines (e.g., IP-10 and MIP-1β), the CC chemokine receptor CCR-7, and immunologically important cell surface molecules (e.g., CD40, CD86, CD54). $DC_{AdVTNF\text{-}\alpha}$ also (i) stimulated stronger allogeneic T cell responses *in vitro* and T cell activation *in vivo*, (ii) displayed enhanced chemotactic responses to MIP-3β *in vitro*, and (iii) trafficked *in vivo* into draining lymph nodes dramatically more efficiently than control DCs. Vaccination of mice with Mut1 peptide-pulsed $DC_{AdVTNF\text{-}\alpha}$ more efficiently stimulated *in vitro* Mut1–specific $CD8^+$ CTL responses and induced solid tumor immunity *in vivo*, relative to DCs cultured in TNF-α.[132]

IL-12 is a heterodimeric cytokine produced by many types of cells, including DCs, macrophages, leukocytes, and keratinocytes.[133] It can enhance NK cell and CTL activities, and plays a key role in the induction of Th1–type immune responses. DCs expressing an IL-12 transgene (and secreting $\approx$25 ng rIL-12/10^6 cells/48 h) can promote enhanced specific anti-tumor CTL responses compared to nontransduced DC.[134] Similarly, intratumoral injection of such IL-12 transduced BM-DCs leads to regression of weakly immunogenic (day 7) established tumors (MCA205, B16, and D122) and to complete regression of established murine transplantable colon adenocarcinomas. This $DC_{IL\text{-}12}$ antitumor effect (and the induction of tumor-specific Th1 responses) is substantially greater than that observed with similarly IL-12–transduced syngeneic fibroblasts or nontransduced BM-DCs. Splenic DCs engineered to express augemented levels of IL-12 also elicit therapeutic antitumor immune responses.[135–137]

Secondary lymphoid tissue chemokine (SLC) is a CC chemokine that is selective in its recruitment of naive T cells and DCs.[139,140] In the lymph node, SLC is believed to play an important role in the initiation of immune responses by co-localizing naive T cells with DCs that are presenting (tumor) antigen. Intratumoral injection of SLC-expressing DCs (DC_{SLC}) results in tumor growth inhibition that is significantly better than observed with either control DCs or SLC alone. Similarly, distant site immunization of tumor-bearing mice with DC_{SLC} that have been pulsed with tumor lysates elicits antitumor responses, whereas control DCs do not. Direct administration into growing B16 melanomas of DC_{SLC} induces a substantial and sustained influx of T cells into the tumor mass, with only transient increases in T cell numbers in the draining lymph node (DLN), suggesting that the DCs are largely retained at the tumor sites, with only a very small proportion of them trafficking to the DLN. Within 24 h, the T cells infiltrating the tumors express the activation marker CD25 and within 7 days they develop IFN-γ-secreting function, in concert with a detectable inhibition of tumor growth. These reports demonstrate that SLC expression by DCs can induce antitumor responses that lead to enhanced antitumor immunity.[141,142]

CD40 ligand (CD40L) is a 33–kDa type II membrane protein that is a member of the tumor necrosis factor (TNF) gene family. It is preferentially expressed on activated $CD4^+$ T cells.[145,146] The receptor for CD40L is the CD40 molecule (a member of the TNF receptor family) expressed on APCs, including DCs. It has recently been reported that CD40–CD40L interactions are essential for CD4+ T cell-dependent activation of DCs.[147–151] Thus, we have investigated the effects on the induction of antitumor immunity of vaccinating mice with DCs engineered to express CD40L. Our data show that transfection of DCs with recombinant adenovirus AdV-CD40L ($DC_{AdVCD40L}$) resulted in activation of the DCs (somewhat as noted above for the $DC_{AdVTNF\text{-}\alpha}$;[134] with up-regulated expression of proinflammatory cytokines (IL-1β and IL-12), chemokines (RANTES, IP-10, and MIP-1α), and immunologically important cell surface molecules (CD54, CD80, and CD86). Our data also demonstrate that $DC_{AdVCD40L}$ are able to stimulate enhanced Mut1–specific CTL responses *in vitro*. Furthermore, vaccination of mice with Mut1 peptide-pulsed $DC_{AdVCD40L}$ induces augmented antitumor immunity *in vivo*, completely protecting eight of eight mice from challenge with high doses of 3LL tumor cells.[152] Moreover, in B16 and CT26 murine tumor models, injection of DC_{CD40L} into established (day 8) subcutaneous

tumors resulted in sustained tumor regression and augmented animal survival. The intratumoral $DC_{AdVCD40L}$–recipient mice expressed tumor-specific CTL responses, and transfer of their spleen cells efficiently protected naive mice against a subsequent tumor challenge. In a distant two-tumor model of metastatic disease, untreated B16 tumors in the right flanks of mice regressed in parallel with DC_{CD40L}–treated left flank tumors.[153] These results support the concept that genetic modification of DCs with a recombinant CD40L adenovirus vector may be another useful strategy for directly activating DCs to be employed for cancer immunotherapy.

OTHER FACTORS THAT AFFECT THE DC VACCINE EFFICACY

There are many factors that can affect the efficacy of DC-based vaccines for cancer therapy. These include the DC doses, and their route(s) and interval(s) of administration, and the choice of antigens, method of antigen loading and the maturation stage of the DCs.

The Presentation Density of Tumor Antigens

The ability of some peptide-pulsed DCs to induce antitumor immune responses depends on the density of the tumor antigens on the DCs, and the relative proportions of the protective antigen(s), relative to irrelevant proteins. It has been shown that peptide preparations from one tumor cell line can confer protection against challenge with the same tumor cell line, while protective immunity to a different tumor line could be induced only if the cells used for peptide preparation presented a high relative proportion of the specific antigen peptide ($LCMV_{33-41}$) in association with MHC class I.[154] T cell activation after DC vaccination is also dependent on the type of antigen and its mode of delivery. Thus, $CEA_{605-613}$–pulsed DCs failed to induce clinical responses, while DCs pulsed with the derivative peptide, 610_D, can prime for antigen-specific immunity, and have shown promising preliminary clinical results.[31] This suggests that it is likely advisable to incorporate multiple peptides or antigens into DC vaccination strategies in order to reduce the risk of possible immune escape of the target tumor cells. This includes the MHC class I-restricted antigens, but also the MHC II-restricted peptides, which may be important for optimizing antitumor effects through recruitment of $CD4^+$ T helper cells into the responses.[121,123]

Carrier Effects and Regulation of Tumor Protein Immunogenicity

Linkage of a foreign carrier protein to a self-tumor antigen, and the addition of foreign helper protein during tumor lysis and peptide-pulsing, can enhance the immunogenicity of pulsed DC vaccines. Thus, vaccination of mice with Id-pulsed DCs induces anti-Id Abs only when the Id protein was modified such that it in effect comprised a hapten-carrier system. DCs pulsed with Id proteins modified to include foreign constant regions plus GM-CSF, or linkage to a KLH carrier protein were increasingly potent in their ability to elicit anti-Id Abs.[155] DCs pulsed with both KLH and tumor lysate mediated enhanced immune priming and rejection of established metastases *in vivo*, and this effect was T cell-dependent. Treatment with DCs pulsed with KLH and mouse TRP-2 peptide resulted in reductions in B16 melanoma metastases; the effect was most pronounced in settings where TRP-2 peptide-pulsed DCs alone are completely ineffective.[156] In another series of experiments, a viral oncogene that carries a series of well-defined K^b/D^b-restricted epitopes (i.e., SV40 large T antigen; T-Ag) was expressed in P815 and Meth-A murine tumor cells in heat shock protein (hsp)-associated or -independent forms. The wild-type T-Ag (wtT-Ag) is expressed without stable hsp association, while the mutant cytoplasmic (cT-Ag) is expressed in stable association with the constitutively expressed, cytosolic hsp73 protein. The authors found that, *in vitro*, remnants from apoptotic wtT-Ag- or cT-Ag-expressing tumor cells could be taken up and processed by immature DC, and the K^b/D^b-binding epitopes (T1, T2/3, and T4) of T-Ag cross-presented to CTL in a TAP-independent manner. However, these DC cross-presented the three T-Ag epitopes more efficiently in the context of hsp73/cT-Ag complexes than in the context of non-hsp-associated (native) T-Ag. This suggests that an association of an oncogene with constitutively expressed cytosolic hsp73 within apoptotic tumor cells facilitates cross-priming by the DC of CTL, both *in vitro* and *in vivo*.[157] However, mature human DCs can boost functionally superior $CD8^+$ T-cell without foreign helper epitopes.[158] Anti-tumor vaccination using DC pulsed with MAGE peptides potently induces a transient MAGE-driven

IFN-γ response that is not influenced by the delivery of additional nonspecific T-cell help.[159]

DCs generated either from $CD34^+$ progenitor cells or from monocytes may differ in their abilities to activate antigen-specific $CD8^+$ T cells, as it has been reported that more antigen-specific $CD8^+$ T cells develop following antigen peptide presentation by $CD34^+$ progenitor cell-derived DCs than develop when monocyte-derived DCs are employed [160].

Host Anti-DC/Tumor Protein CTL Responses

One potential complication that could arise as a consequence of repeated patient vaccination with tumor peptide-carrying DCs is that these cells could themselves become targets of the host s CTL response. Indeed, weekly administration of peptide–pulsed DCs has been shown to lead to a diminishing CTL activity,[161] and such CTL-mediated clearance of antigen-loaded DCs has a notable effect on immune responses *in vivo*. Antigen-specific $CD8^+$ T cells may fail to respond to DCs loaded with antigen if the DCs are targets of a pre-existing CTL response, so that repeated immunization with $LCMV_{33-41}$–loaded DCs does not lead to enhanced CTL responses against LL-LCMV challenge. Nevertheless, repeated DC injections may be effective in maintaining effector function in "memory" $CD8^+$ T cells that may have lost activity due to suboptimal presentation of antigen in the context of tumor tissue. The function of such CTL-mediated clearance of antigen-loaded DCs may be that of a negative feedback mechanism designed to limit the activity of DCs within the lymph node. This concept of DC clearance has important implications for the design of DCs-based immunotherapy protocols. For example, repeated immunizations at short intervals with DCs bearing MHC class I-bound tumor peptide may quickly become ineffective in enhancing responses to this antigen as the peptide-loaded DCs are eliminated by the growing peptide-specific CTL response.[162]

Route of DC administration

The route of DC administration has some influence on DC migration to lymphoid tissues and thus on the efficacy of the DC vaccine. Syngeneic bone marrow-derived, tumor lysate-pulsed DCs administered directly into the lymph nodes generated more potent protective antitumor immunity than analogous s.c. or i.v. DC immunization.[163] After a single vaccination with 1×10^6 OVA-pulsed DC2.4 cells, tumors were rejected in the mice given their DC vaccinations i.d. (three of four mice), s.c (three of four mice), or i.p. (one of four mice). Double vaccinations further enhanced the antitumor effect in the i.d. and s.c., but not the i.v., group.[164] In a clinical trial, three patient cohorts were immunized with antigen-pulsed DC by i.v., i.d., or intralymphatic (i.l.) injection, and all patients developed antigen-specific T cell responses following immunization. However, induction of IFN-γ production was seen only with the i.d. and i.l. routes of administration and, consistent with this apparent Th1 (i.e., IFN-γ response) antigen-driven IL-4 expression was not seen, regardless of the route. In a human trial, five of nine patients immunized by the i.v. route developed antigen-specific antibodies, compared with one of six and two of six for groups treated via the i.d. and i.l. routes, respectively. These results suggest that, while activated DC can prime for the induction of specific immune responses regardless of route by which they are delivered, the quality of the responses can be affected by the DC delivery route.[165] DCs injected intravenously accumulate largely in the spleen, whereas those injected subcutaneously preferentially home to the T cell areas of the draining lymph nodes. It might be predicted *a priori* that this could substantially impact the development of tumor-specific responses. Thus, in an autologous B16 melanoma model system in which TRP2 peptide-loaded DCs were used for vaccination, increased antitumor cytotoxic T-cell reactivity, delayed tumor growth, and improved survival were more evident after s.c. DC vaccination, than after i.v. vaccination. These data demonstrated that optimal induction of antitumor reactivity driven by the autologous melanocyte differentiation antigen TRP2–derived peptide correlated with the preferential accumulation of DCs in the T cell-rich areas of the lymph nodes.[166]

DC Maturation Status

The maturation status of DCs used for antitumor vaccination has a decisive influence on the outcomes of peptide-pulsed DC strategies. Terminally differentiated mature DCs are necessary for the induction of optimal tumor antigen-specific CTL responses; DCs cultured in the presence of CD40L or LPS are more mature relative to DCs cultured with TNF or Flt3 ligand.[167] We have re-

cently investigated in some detail the impact of the relative maturation levels of DCs on their phenotypes. DC maturation driven by LPS lead to significantly heightened expression of the DC s antigen-presenting machinery (e.g., CD54, CD80, CD86) and numerous cytokines and chemokines/chemokine receptors (i.e., Flt-3L, G-CSF, IL-1β and –1β, IL-6, IL-12, CCL-2, -3, -4, -5, -17, and -22, MIP-2, and CCR7). These LPS-treated mature DCs are also significantly better at inducing effector T cell responses *in vitro* than less mature or immature DCs (generated by culture in GM-CSF/IL-4– or GM-CSF-containing medium, respectively)[154] Furthermore, mice vaccinated with these mature DCs better survived challenge with 3LL tumor cells (8 of 8 survivors) than did mice vaccinated with less mature (3 of 8 survived) or immature (0 of 8 survivors) DCs. These data clearly underscore the potentially critical nature of employing DCs of full maturity for DC-based antitumor vaccination strategies.[168]

SUMMARY

In summary, DC-based cancer vaccines comprise a particularly attractive approach to cancer immunotherapy. In animal models, vaccination with DCs simply pulsed with tumor peptides, lysates, or RNA, or loaded with apoptotic/necrotic tumor cells induce significant antitumor CTL responses and antitumor immunity. A step up from this, DCs expressing transgenes encoding tumor antigens are more potent primers of antitumor immunity both *in vitro* and in animal models *in vivo* than DCs pulsed with tumor peptides. Engineering DCs to express relevant cytokines or chemokines further improves their maturation status, and thereby their capacity to migrate to secondary lymphoid organs, stimulate tumor-specific T cell responses, and induce antitumor immunity *in vivo*. We have also discussed many factors that can importantly influence DC vaccination efficacy, including, (i) the type of tumor peptides used for pulsing DCs and their expression density on these cells, (ii) the timing and intervals of DC vaccination, (iii) the route of vaccination, and (iv) the DC maturation status. In conclusion, we predict that the next decade of DC vaccine research will continue to provide highly relevant insights into our ultimate objective, the successful treatment of patients with tumors of all kinds.

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